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PLGA microparticles: possible vehicles for topical drug delivery

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Abstract

Distribution of PLGA-microparticles in porcine skin, after its topical application, was studied in vitro using microparticles containing rhodamine as a fluorescent probe. PLGA-microparticles loaded with rhodamine were prepared using a solvent evaporation technique. Skin distribution of fluorescent microparticles was performed, by horizontal and vertical slicing of frozen skin. Fluorescence photomicrographs revealed that PLGA-microparticles could penetrate through the stratum corneum and reach the epidermis. However, permeation experiments showed that these microparticles were not able to reach the receptor compartment of the diffusion cells, even in a period of 24 h. The carriers described in this work could be used as vehicles for topical drug delivery, in order to obtain a sustained drug release into the skin, improving therapy by reduction of time intervals between doses.

Keywords: PLGA-microparticles; Rhodamine; Porcine skin; Topical drug delivery; Skin slicing

In recent years, microparticulate drug delivery systems have emerged as one of the most promising strategies to achieve site-specific drug delivery (Berthold et al., 1998).

These systems have been extensively utilised for oral and parenteral administration, and they could be useful to deliver several drugs into the skin. To supply the skin with a drug, over a prolonged period of time, and to reduce its systemic absorption, a sustained drug release would be necessary (Jenning et al., 2000a). In this way, several authors have used these systems for topical delivery of different drugs; such as retinol (Rösseler et al., 1994), 5-fluorouracil-ethyl (Ghorab et al., 1990) and some allergenic substances (Brosse et al., 2000). Also, coated titanium dioxide microparticles are commonly used as UV filter substances in commercial sunscreen products (Lademann et al., 1999).

The aim of this work was to study the penetration and distribution of poly(D,L-lactic-co-glycolic acid) PLGA-microparticles, in porcine skin, after its topical application. Biodegradable PLGA polymers are normally used in the preparation of these carriers (Gabor et al., 1999).

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Poly(D,L-lactid-co-glycolid) Resomer® RG 502 H 12 000 Da MW has been purchased from Boehringer Ingelheim (Ingelheim, Germany). Polyvinyl alcohol (PVA) 115 000 MW was supplied by BDH (Poole, England) and dichloromethane was provided by Prolabo (Fontenay, France). Rhodamine B Base (Sigma Chemical, St. Louis, USA) was employed as a fluorescent probe.

PLGA microparticles containing rhodamine were prepared by the O/W solvent evaporation technique. Four milligrams of rhodamine were dispersed in a 16% (w/v) polymer solution in dichloromethane. The resulting dispersion was added to 30 ml of a 0.5% PVA solution and homogenised using an ultraturrax® (Euro Turrax T20b IKA Labortechnik, Staufen, Germany) for 1 min. This mixture was then stirred at 25 °C for at least 3 h until complete solvent evaporation. Microparticles were collected by centrifugation (3000 rpm for 10 min, Biofuge stratus Heraeus Instruments, Hanau, Germany), washed three times with distilled water, freeze-dried for 48 h (Virtis Genesis 12 EL, Gardines, NY) and stored at 4 °C.

For morphological examinations, microparticles were analysed with a Scanning Electron Microscopy (SEM; Scanning digital electron microscope DSM-940A, Zeiss, Germany) after being fixed on a sample support and gold metallized.

Microparticles diameter was measured by laser diffractometry, using a Matersizer S (Malvern Instruments, Worcestershire, UK). The average particle size was expressed as the volume mean diameter in micrometers.

The encapsulation of rhodamine was measured fluorometrically, with excitation and emission wavelengths of 545 and 583 nm, respectively (Perkin Elmer LS50, Spain) after breaking the microparticles with dichloromethane.

Porcine ears were obtained from the local slaughterhouse (Pamplona, Spain) and after cleaning, the outer region of the ear was employed. The skin was dermatomed (AESCULAP®, Tuttlingen, Germany) and stored at −20 °C. The thickness of skin samples was 1.2 mm.

In permeation studies, Franz diffusion cells (FDC-400, Grown Glass Company, Somerville, NY) (1.76 cm^2 surface area) were used. Skin was mounted on the diffusion cells with the stratum corneum facing the donor compartment. Eleven millilitres of phosphate buffer solution (pH 7.4) was used as the receptor medium and 1 ml of microparticles containing rhodamine (0.3 mg) was placed on the skin surface, in the donor compartment. Also 400 μl aliquots were collected from the receptor side of the diffusion cells at designated time intervals (6, 12 and 24 h). The receptor medium was maintained at 37 ± 1 °C and stirred at 600 rpm using magnetic stirring bars during 24 h. After the in vitro experiments, the skin was removed (1.76 cm^2) and carefully cleaned with distilled water, frozen in liquid N_2 and cut with a cryomicrotome (2800 Frigocut E, Reichert-Jung, Germany) to obtain 3 μm slices for vertical slicing and 10 μm for horizontal cutting. Slices were observed with a fluorescence microscope (Nikon EF-D Mercury Set, Badhoevedorp, The Netherlands).

The resulted microspheres were in the size range of 1–10 μm with a volume mean diameter of 4.61 ± 0.8 μm. By SEM, the microspheres appeared to be spherical and nonaggregated. The encapsulation of rhodamine, measured fluorometrically was $60 \pm 5\%$.

In order to study the penetration of the microparticles, through the porcine skin, 1 ml of fluorescent microparticles (0.3 mg) was placed on the skin surface in the donor compartment and the permeation experiment was carried out. The skin membrane was observed, by light microscopy, before and after the permeation experiments to confirm its integrity. No skin structural alterations were seen after permeation experiments. Also 400 μl aliquots were collected from the receptor side of the diffusion cells, at designated time intervals (6, 12 and 24 h) and no rhodamine was detected in any case. These results showed that PLGA-microparticles were not capable to permeate through porcine skin, even in a period of 24 h.

After permeation experiments, the skin was cut in vertical and horizontal slices to observe the microparticles distribution in the skin. Untreated

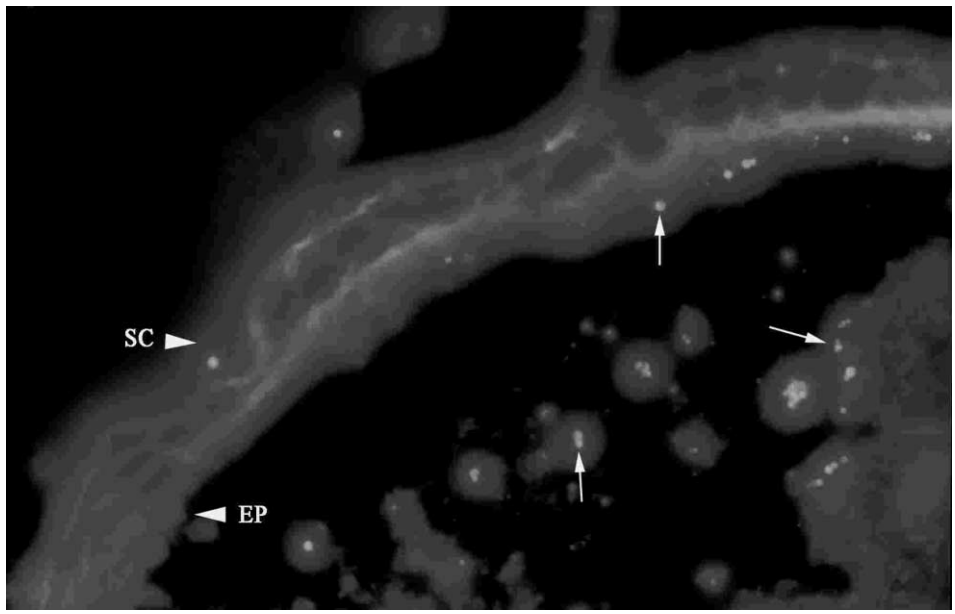


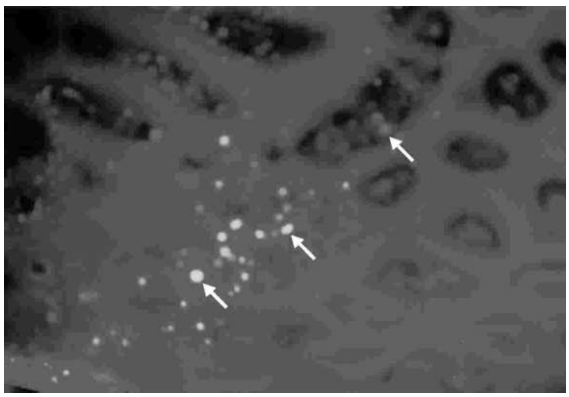
Fig. 1. Fluorescence photomicrograph of vertical slicing of porcine skin, after the application of rhodamine loaded-microparticles (arrows) (24 h). SC, stratum corneum; EP, epidermis.

skin was used to evaluate the intrinsic fluorescence of the tissue. As a control, permeation experiments and skin slicing were also carried out with a rhodamine solution.

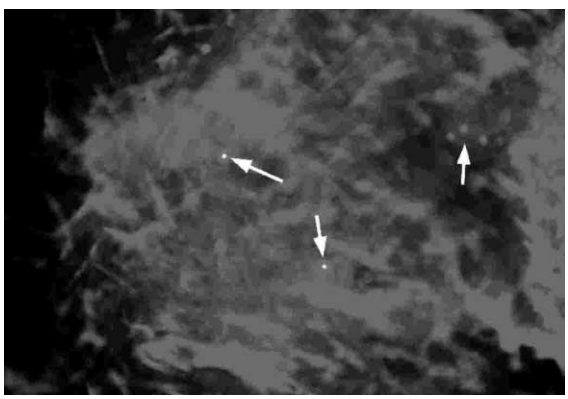
A representation of a vertical slicing after microparticles application is given in Fig. 1. The microparticles were clearly visualised in the stratum corneum, epidermis and dermis. When the rhodamine solution was applied to the skin, no fluorescent microparticles were observed but only the red background corresponding to the free probe.

Horizontal slicing from different depth showed a larger accumulation of microparticles in the stratum corneum. It could be noticed that the biggest microparticles did not penetrate the skin and remained on the stratum corneum surface. In addition, the number of microparticles decreased when the depth of the slices was increased. Jenning et al. (2000b) reported, that in the porcine skin, the upper 100 μm represent mainly the stratum corneum and upper layers of viable epidermis, between 100 and 200 μm consists basically of viable epidermis and the dermis is located from 200 to 500 μm. Consequently, at 120 μm (which represents viable epidermis) (Fig. 2A), the number of microparticles observed was relatively high, conversely at 400 μm (dermis) (Fig. 2B) we can hardly see any microparticles. Finally, when the depth of the slice was 500 μm, no microparticles were found, but only free rhodamine.

Previous papers have explored the use of microparticles for topical application, Rolland et al. (1993) reported that adapalene loaded microspheres (5 μm mean diameter) were specifically targeted to the follicular ducts and did not penetrate via the stratum corneum. From our results, we can conclude that the microparticles developed in this study can effectively get into the skin through the stratum corneum and reach the epidermis in a relatively high number, although the biggest ones remained in the skin surface. Consequently these carriers seem to be promising systems for drug topical administration.



A



B

Fig. 2. Photomicrographs of horizontal slicing of skin from different depths, after the application of rhodamine loaded-microparticles (arrows): (A) 120 μm (B) 400 μm.

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